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Risk Factors for Necrotizing Enterocolitis: A Prospective Multicenter Case-Control Study

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Keywords
Neonatology · Sepsis · Formula feeding · Risk factors · Mortality · Antibiotics

Abstract
Background: The identification of independent clinical risk factors for necrotizing enterocolitis (NEC) may contribute to early selection of infants at risk, allowing for the development of targeted strategies aimed at the prevention of NEC. ***Objective:*** The objective of this study was to identify independent risk factors contributing to the development of NEC in a large multicenter cohort. ***Methods:*** This prospective cohort study was performed in 9 neonatal intensive care units. Infants born at a gestational age ≤30 weeks were included. Demographic and clinical data were collected daily until day 28 postnatally. Factors predictive of the development of NEC were identified using univariate and multivariable analyses in a 1:5 matched case-control cohort. ***Results:*** In total, 843 infants (56 NEC cases) were included in this study. In the case-control cohort, univariate analysis identified sepsis prior to the onset of NEC and formula feeding to be associated

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with an increased risk of developing NEC, whereas the administration of antibiotics directly postpartum was inversely associated with NEC. In a multivariable logistic regression model, enteral feeding type and the number of days parenterally fed remained statistically significantly associated with NEC, whereas the administration of antibiotics directly after birth was associated with a lower risk of developing NEC. ***Conclusions:*** Formula feeding and prolonged (duration of) parenteral feeding were associated with an increased risk of NEC. Contrary to expectations, the initiation of treatment with antibiotics within 24 h after birth was inversely associated with NEC.

Introduction

Necrotizing enterocolitis (NEC) is the most common severe gastrointestinal disease in infants born preterm. A new guideline on perinatal treatment of infants born extremely preterm was implemented in the Netherlands in 2010, lowering the border of viability to support active treatment from 25 to 24 weeks' gestation. This resulted in an increase in NEC incidences (16% in infants born at a gestational age [GA] <28 weeks) and associated mortality [1].

The etiology of NEC is considered multifactorial, but the contribution of individual risk factors remains yet to be elucidated [2]. Several studies, mostly retrospective in design, have aimed to identify independent risk factors for NEC. Prematurity and low birth weight were the most consistently identified risk factors for the development of NEC [3]. Other reported risk factors include the administration of bovine-origin formula [4], low Apgar scores [5], small for GA [6], treatment of patent ductus arteriosus (PDA) [7], erythrocyte transfusions [8], and nosocomial infections [9]. Studies on potential effects of postnatal antibiotics on NEC incidence have shown conflicting results [10]. The identification of clinical factors contributing to the development of NEC may allow for the selection of neonates at risk for NEC and could contribute to the development of strategies aimed at the prevention and early treatment of NEC. Therefore, the aim of this study was to identify independent variables that are associated with the development of NEC. The daily collection of a wide variety of clinical variables, including exposure to antibiotics, allowed to explore their potential role in a detailed prospective manner.

Patients and Methods

Patients

This prospective cohort study, including infants born at a GA ≤30 weeks, was conducted between October 2014 and January 2017 at 2 level III and 7 level IV neonatal intensive care units (NICUs) in the Netherlands and Belgium (online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000489677). In that study, potential diagnostic biomarkers for NEC and sepsis are investigated [11, 12]. None of the participating NICUs administered probiotics routinely. This study was approved by the local Institutional Review Boards of all 9 medical centers (2014.386 amendment A2016.363). Informed consent was obtained from all parents.

Data on GA, birth weight, gender, delivery mode, multiple births, preterm premature rupture of membranes (PPROM) (≥24 h before delivery), and Apgar scores were collected. Additional clinical data, including treatment of a significant PDA, diagnosis of NEC or sepsis, including causative organism, administration of antibiotics, transfusions with erythrocytes, use of central catheter, and parenteral and enteral feeding practices were prospectively collected. Data collection was ceased in case of transfer to another hospital. Infants were excluded in case of a missing or incomplete medical file.

Definitions

NEC cases were defined as infants diagnosed with NEC stage ≥IIA (Bell's classification). NEC cases were independently reviewed by two experts (T.G.J.d.M. and H.J.N.), and consensus was met in all cases. Infants meeting all 3 Vermont Oxford criteria for sepsis were identified as sepsis cases, including (1) clinical symptoms of generalized infection (e.g., temperature instability, apnea, hemodynamic instability); (2) isolation of a pathogen from a blood culture; and (3) treatment with antibiotics for ≥5 days directed to this pathogen [13]. A hemodynamically significant PDA was defined as an echocardiographic confirmed PDA for which pharmacological (ibuprofen, indomethacin) or surgical treatment was initiated.

Exposure to antibiotics was defined in two ways. The first definition, describing the duration of administrating antibiotics initiated within 24 h after birth, was categorized into (1) 0 days, (2) ≤3 days, or (3) >3 days. In addition, types of antibiotics were also noted. The second definition described the cumulative number of days a patient was on any treatment with antibiotics. Exposure to central lines and red blood cell transfusion were noted as cumulative number of days any central line was present or erythrocytes were administered, respectively.

For enteral feeding types, we defined the following subgroups: (1) breast milk fed, defined as the average daily enteral feeding volume consisting of ≥80% breast milk, including donor milk; (2) formula fed, defined as the average daily enteral feeding volume consisting of ≥50% formula; and (3) a combination of both formula and breast milk, including infants not meeting the criteria of the first two subgroups. Full enteral feeding was defined when for at least two consecutive days no additional parenteral feeding (amino acids or lipids) was administered. Exposure to parenteral feeding was noted as the cumulative number of days any nutritional solution (lipids or amino acids) was administered parenterally. Increments in feeding volumes during the first 7 days postnatally were defined as the daily increase in enteral feeding volume relative to the birth weight (mL/kg/day).

Table 1. Inclusions per participating center

Center	Inclusion period, months	Total inclusions, *n* (%)	Incidence of NEC, *n* (%)	Median age at the development of NEC, days [IQR]
1	14	52 (6.2)	2 (3.8)	8.5
2	28	179 (21.2)	8 (4.5)	14.5 [6.5–19.8]
3	11	104 (12.3)	5 (4.8)	15 [9–23.5]
4	17	90 (10.7)	5 (5.6)	16 [13.5–22.5]
5	4	17 (2.0)	1 (5.9)	10
6	27	114 (13.5)	8 (7.0)	14 [7.5–22.3]
7	28	165 (19.6)	14 (8.5)	9.5 [7.8–17.3]
8	27	65 (7.7)	6 (9.2)	15 [12–21.8]
9	10	57 (6.8)	7 (12.3)	9 [6–10]

IQR, interquartile range; NEC, necrotizing enterocolitis.

Statistical Analysis

We performed two statistical analyses on the cohort. First, during the entire inclusion period of 28 days, demographic and clinical variables from all infants with NEC were compared with the variables from all infants without NEC. Second, each NEC case was matched to 5 controls, defined as infants without NEC. The matching procedure was based on GA (maximum difference of 3 days), birth weight (maximum difference of 400 g), and postnatal age (total number of hospital days prior to NEC in matched cases). In this nested case-control cohort, variables of interest were collected for both the cases and matched controls from birth up to postnatal age of NEC diagnosis (t_0). For example, if a case developed NEC on postnatal day 12, data was collected from both the case and matched controls from birth up to the postnatal age of 12 days.

Statistical analysis was performed with Statistical Package for the Social Science (SPSS) version 22.0. Predictive factors were identified by univariate analysis and corresponding *p* values, odds ratios and 95% confidence intervals were noted. Variables with a two-sided *p* value ≤0.20 were included in the multivariable logistic regression model. This model was constructed using the forward stepwise selection method, ultimately including statistically significant variables, defined as having a *p* value <0.05. Since center-specific incidence rates were relatively low, birth center was excluded from the regression models.

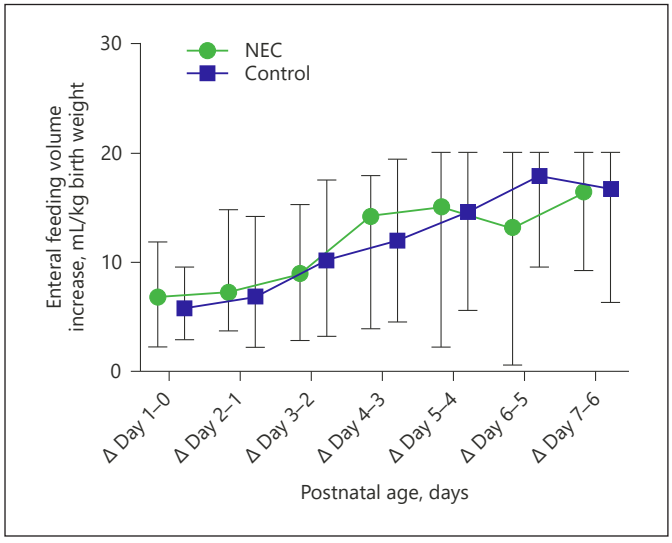

Fig. 1. Median daily enteral feeding volume increase during the first 7 postnatal days. Median daily enteral feeding volume increase (mL/kg/day) with corresponding interquartile ranges are shown for both cases (dots) and controls (squares) within the 1:5 case-control cohort.

Results

Total Cohort

In total, 843 preterm infants were included during the study period. Fourteen (1.7%) were excluded based on missing or incomplete medical files. Of the 829 remaining infants, 56 (6.8%) developed NEC within the first 28 postnatal days. NEC was diagnosed at a median postnatal age of 12 days [IQR: 8.3–18 days]. The distribution of severity was stage IIA in 19 (33.9%) cases, IIB in 16 (28.6%) cases, IIIA in 8 (14.3%) cases, and IIIB in 13 (23.2%). Table 1 depicts incidence rates per center (displayed encoded). An overview of the demographic and clinical characteristics of the cases versus controls during the entire inclusion period of 28 days is depicted in Table 2. In the NEC population, 21.4% of the infants died during the follow-up period, compared to 4.8% of the controls.

Case-Control Cohort

Overall, 56 cases and 280 matched controls were included in the case-control analysis. Outcomes of the uni-

Table 2. Demographic and clinical characteristics of all included subjects per study group during the entire inclusion period of 28 days

	NEC cases (n = 56)	Controls (n = 773)	p value	Unadjusted OR [95% CI]
Median gestational age [IQR], weeks+days	26+6 [25+2 to 27+6]	27+6 [26+3 to 29+0]	<0.001	0.951 [0.929–0.974]
Mean birth weight ± SD, g	896±241	1,041±274.3	<0.001	0.998 [0.997–0.999]
Birth season (fall–winter)[a], n (%)	31 (56)	409 (53)	<0.723	1.10 [0.640–1.90]
Male gender, n (%)	28 (50)	408 (53)	<0.687	0.895 [0.520–1.54]
Delivery mode (vaginal delivery), n (%)	29 (52)	354 (46)	<0.391	1.27 [0.737–2.18]
Multiple births, n (%)	19 (34)	249 (32)	<0.791	1.08 [0.609–1.92]
PPROM, n (%)	9 (16)	169 (22)	<0.324	0.691 [0.332–1.44]
Median Apgar [IQR]				
1 min	5 [3–8]	6 [4–7]	<0.517	0.962 [0.855–1.08]
5 min	8 [7–9]	8 [7–9]	<0.886	1.01 [0.859–1.19]
Sepsis, n (%)	37 (66)	208 (26)	<0.001	5.29 [2.98–9.41]
PDA, n (%)	31 (55)	255 (33)	<0.001	2.51 [1.45–4.35]
Central line exposure, n (%)	54 (98)	618 (81)	<0.011	13.1 [1.80–95.7]
Median duration of central line exposure [IQR], days	15 [9–26]	8 [5–12]	<0.001	1.14 [1.104–1.19]
RBC transfusion exposure, n (%)	54 (96)	455 (60)	<0.001	18.4 [4.45–76.0]
Median duration of RBC transfusion [IQR], days	3 [2–5]	1 [0–2]	<0.001	1.44 [1.29–1.60]
Antibiotic exposure, n (%)	56 (100)	741 (96)	<0.998	∞
Median duration of antibiotic exposure [IQR], days	17 [10.3–21]	7 [4–13]	<0.001	1.15 [1.10–1.19]
Enteral feeding type[b], n (%)				
Breast milk fed	22 (46)	491 (69)	<0.003	Reference
Formula fed	11 (23)	116 (16)	<0.051	2.11 [0.998–4.49]
Combination	15 (31)	103 (15)	<0.001	3.25 [1.63–6.48]
Achieved full enteral feeding[c], n (%)	38 (68)	631 (92)	<0.001	0.184 [0.099–0.344]
Median duration of parenteral feeding before being fully enteral fed [IQR], days	13 [13.3–27.8]	10 [8–12]	<0.001	1.19 [1.12–1.27]
Median duration of parental feeding [IQR], days	20 [9–17.3]	10 [8–13]	<0.001	1.24 [1.19–1.30]
Mortality, n (%)	12 (21)	36 (5)	<0.001	5.58 [2.71–11.5]
Median age at death [IQR], days	12 [8.3–17]	14 [8–18]	<0.634	0.977 [0.889–1.08]

CI, confidence interval; IQR, interquartile range; OR, odds ratio; PPROM, preterm premature rupture of membranes; SD, standard deviation.
[a] October up to March are defined as fall-winter [b] Variables were not retrievable from the medical records of one participating center (n = 71 missing values). [c] Variables were not retrievable from the medical records of one participating center (n = 87 missing values).

variate analysis are depicted in Table 3, demonstrating both sepsis and formula feeding prior to clinical presentation to be associated with an increased risk of developing NEC, whereas initiating of the administration of antibiotics within 24 h after birth was inversely associated with NEC. In total, 23.2% (n = 13) of the cases did not receive antibiotics within 24 h after birth compared to 9.6% (n = 27) of the controls (Fig. 1a). Figure 1b depicts the types of antibiotics administered directly postnatally per study group. Daily enteral feeding increments per study group are depicted in Figure 2.

In the multivariable model, including all 8 variables with a p value ≤0.20 (online suppl. Table 2), treatment with antibiotics administrated directly postnatally (p = 0.004) remained inversely associated with the development of NEC. More specifically, empiric use of antibiotics for a prolonged period of time (>3 days) was associated with decreased odds of developing NEC (OR 0.227 [95% CI 0.079–0.648]; p = 0.006), whereas the odds for infants receiving antibiotics for ≤3 days maximally were: OR 0.213 [0.084–0.544]; p = 0.001. Concerning formula feeding, the odds associated with the development of NEC increased for formula-fed infants (OR 3.36 [1.40–8.03]; p = 0.006) in the multivariable analysis compared to univariable analysis. In addition, the number of days infants received parenteral feeding prior to t_0 (OR 1.19 [1.07–1.31]; p = 0.001) was also associated with an increased odds for developing NEC (Table 3).

Discussion

In this matched prospective multicenter cohort study, we aimed to identify demographic and clinical factors that preceded the development of NEC in preterm infants. Multivariable logistic regression modeling demon-

Table 3. Characteristics of the NEC infants and the 5 matched controls per NEC case in the period preceding NEC diagnosis (t_0)

	NEC (n = 56)	Matched controls (n = 280)	p value	Univariate analysis OR [95% CI]	p value	Multivariable analysis OR [95% CI]
Median gestational age [IQR], weeks+days	26+6 [25+3 to 27+6]	26+6 [25+3 to 27+6]	0.936	1.00 [0.974–1.03]		
Mean birth weight ± SD, g	896±241	892±314	0.904	1.00 [0.999–1.00]		
Birth season (fall–winter)[a], n (%)	31 (55)	143 (51)	0.558	1.19 [0.667–2.12]		
Male gender, n (%)	28 (50)	139 (50)	0.961	1.01 [0.571–1.80]		
Delivery mode (vaginal delivery), n (%)	29 (52)	133 (48)	0.558	1.19 [0.669–2.11]		
Multiple births, n (%)	20 (36)	87 (31)	0.496	1.23 [0.675–2.25]		
PPROM, n (%)	9 (16)	66 (24)	0.217	0.618 [0.288–1.33]		
Median Apgar [IQR]						
1 min	5 [3–8]	5 [3–7]	0.403	1.06 [0.929–1.20]		
5 min	8 [7–9]	7 [6–8]	0.186	1.13 [0.943–1.35]		
Sepsis prior to t_0, n (%)	19 (34)	55 (20)	0.020	2.10 [1.12–3.93]		
Gram negative	3 (16)	14 (26)	0.549	0.549 [0.139–2.17]		
Gram positive	5 (26)	10 (18)	0.450	1.61 [0.470–5.50]		
CoNS	12 (63)	34 (62)	0.917	1.06 [0.360–3.12]		
Fungi	1 (5)	1 (2)	0.446	3.00 [0.178–50.5]		
Sepsis within 24 h from t_0, n (%)	27 (48)	12 (4)	<0.001	20.8 [9.53–45.4]		
Gram negative	15 (56)	4 (33)	0.206	2.50 [0.604–10.3]		
Gram positive	4 (15)	2 (17)	0.882	0.870 [0.136–5.55]		
CoNS	10 (37)	7 (58)	0.221	0.420 [0.105–1.68]		
Fungi	0	0	n.a.	n.a.		
PDA, n (%)	24 (43)	114 (41)	0.766	1.09 [0.611–1.95]		
Ibuprofen last administered prior to t0	22 (92)	105 (92)	0.943	0.943 [0.190–4.67]		
Time between last dose and t_0 [IQR], days	3.5 [0–6.8]	3 [0–9]	0.434	0.971 [0.898–1.05]		
Central line exposure prior to t_0, n (%)	50 (91)	251 (90)	0.776	1.15 [0.427–3.13]		
Median duration of central line exposure [IQR], days	8.5 [6–12]	8 [5–10.8]	0.473	0.988 [0.957–1.02]		
RBC transfusion exposure prior to t_0, n (%)	37 (66)	162 (58)	0.255	1.42 [0.777–2.59]		
Median duration of RBC transfusion [IQR], days	2 [1–3]	2 [1–3]	0.924	1.01 [0.811–1.26]		
Time between last RBC transfusion and t_0 [IQR], days	3 [0.8–7]	4 [1–7]	0.621	0.980 [0.903–1.06]		
Infants with RBC transfusion within 48 h of t_0, n (%)	12 (32)	42 (26)	0.455	1.34 [0.622–2.89]		
Antibiotic exposure prior to t_0, n (%)	51 (91)	269 (96)	0.119	0.417 [0.139–1.25]		
Median antibiotic exposure [IQR], days	6.5 [3.3–10.8]	6 [3–9]	0.407	1.03 [0.966–1.09]		
Time between last administered antibiotics and t_0 [IQR], days	3 [0–6]	2 [0–6]	0.536	0.981 [0.924–1.04]		
Postpartum antibiotics, n (%)						
No antibiotics	13 (23)	27 (10)	0.021	Reference	0.004	Reference
1–3 days of antibiotics	28 (50)	170 (61)	0.007	0.342 [0.158–0.741]	0.001	0.213 [0.084–0.544]
>3 days of antibiotics	15 (27)	83 (30)	0.026	0.375 [0.159–0.887]	0.006	0.227 [0.079–0.648]
Enteral feeding type, n (%)						
Breast milk fed	27 (56)	169 (65)	0.110	Reference	0.015	Reference
Formula fed	13 (27)	38 (15)	0.046	2.14 [1.01–4.53]	0.006	3.36 [1.40–8.03]
Combination	8 (17)	18.9 (20)	0.895	0.945 [0.405–2.20]	0.824	0.902 [0.364–2.23]
Mean enteral feeding volume increase during first 7 postnatal days ± SD, mL/kg/day	10.1±5.1	11.2±5.3	0.162	0.962 [0.911–1.02]		
Achieved full enteral feeding prior to t_0, n (%)	30 (54)	106 (41)	0.094	1.64 [0.919–2.94]		
Median time of parental feeding [IQR], days	9 [8–13]	9 [7–11]	0.117	1.07 [0.983–1.17]	0.001	1.19 [1.07–0.1.31]
Mortality, n (%)	13 (23)	17 (6)	<0.001	4.67 [2.12–10.3]		
Median age at death [IQR], days	12 [8–7]	16 [9–17.5]	0.315	0.930 [0.808–1.07]		

CI, confidence interval; IQR, interquartile range; OR, odds ratio; PPROM, preterm premature rupture of membranes; RBC, red blood cell; SD, standard deviation; t_0, day of NEC onset.
[a] October up to March are defined as fall–winter.

strated only 2 independent variables to be associated with an increased risk of NEC: administration of predominantly formula feeding and the cumulative number of parental feeding days. Remarkably, administration of any antibiotics initiated *within 24 h after birth* was associated with a reduced risk of NEC.

NEC incidence in this cohort was 6.8%. Although this reflects incidence rates described in larger international cohorts [14], it is considerably lower than that of a previous study in the Netherlands [1]. This apparent discrepancy may at least partly be explained by the limited follow-up period of 28 days. In the current study, 21.4% of the infants who developed NEC died prior to reaching the age of 28 days. Although this mortality rate is in line with available literature [15], NEC-associated mortality in this cohort may be higher due to the limited follow-up time of 28 days.

As expected, our study confirmed the significant role of low GA and birth weight as risk factors for the development of NEC. During the 28 days of follow-up, preterm infants with NEC were more exposed to antibiotics, central lines, and transfusions with erythrocytes, and they were less likely to reach full enteral feeding at the end of the inclusion period, reflecting the high morbidity rate of NEC.

An association between a hemodynamically significant PDA and NEC was demonstrated in the overall cohort but not in the case-control analysis. Presumably, the strong association between GA and PDA may be the explanation of this detected difference in the overall cohort, since the cases were born at a significantly lower GA. The currently observed absence of an association between transfusions with erythrocytes and NEC is supported by findings in a recent prospective observational study [16].

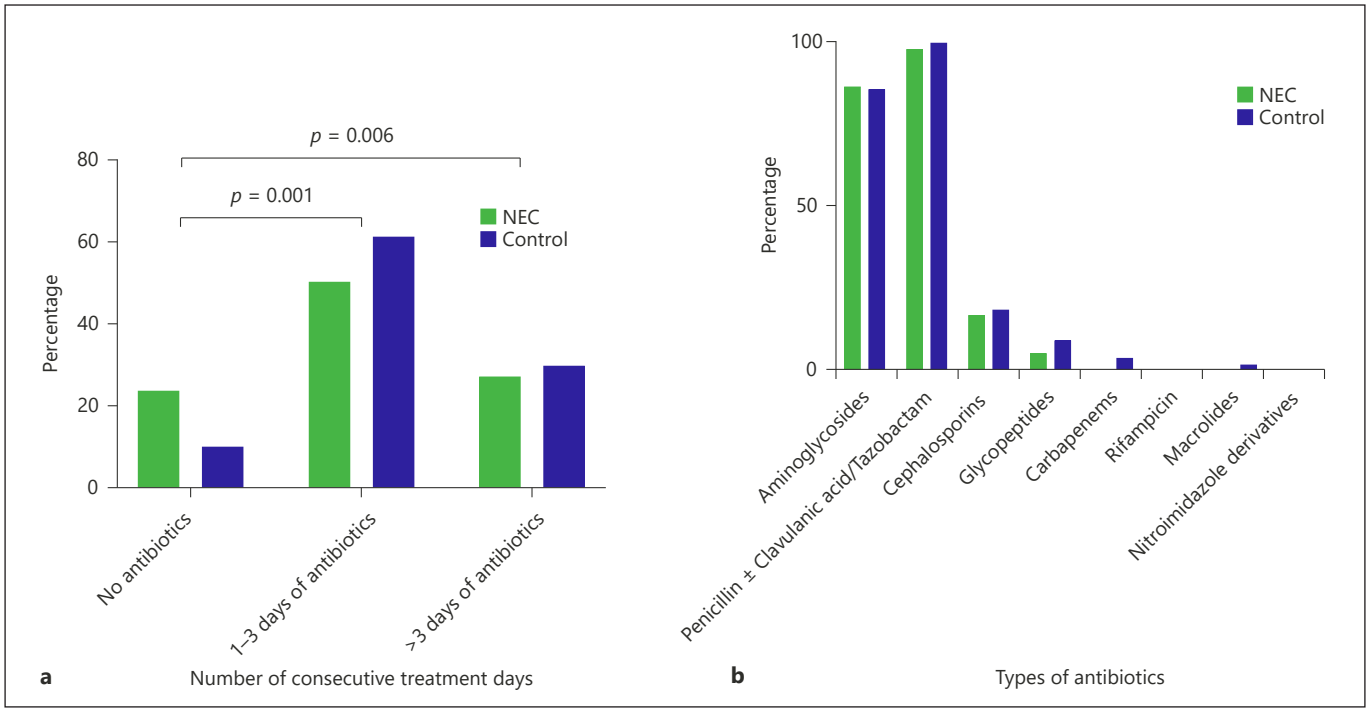

Fig. 2. Administration of antibiotics initiated within 24 h after birth in the matched case-control cohort. **a** Difference in number of consecutive days of treatment with any antibiotic. **b** Difference in types of administered antibiotics during the consecutive number of treatment days.

The initiation of antibiotics within 24 h after birth was inversely associated with the development of NEC. In contrast, Cotton et al. [17] demonstrated an increased risk of developing NEC with increasing treatment days. However, and in contrast to the current study, infants not exposed to antibiotics within 72 h postnatally were excluded. Our results are in line with the results of an observational single-center study describing a reversed association between early initiation of treatment with antibiotics (within 48 h after birth) and development of NEC [18]. In addition, a recent randomized controlled trial on preterm piglets demonstrated sustained administration of antibiotics initiated directly after birth to be protective against NEC [19]. Moreover, in a recent study by Heida et al. [20], a NEC-associated gut microbiota composition was already observed to be present in the meconium. These observations, in combination with the absence of any association between the development of NEC and the total number of days treated with antibiotics suggest that, in particular, initial intestinal colonization plays an essential role in the pathogenesis of NEC. Based on these findings, it could be hypothesized that microbial manipulation, for example through the administration of targeted probiotics or antibiotics, may serve as an effective preventive strategy against NEC. Yet, the observational character of this study hampered the ability to explore any causal relationship between early colonization and the development of NEC. To prove any causality, future studies should focus on obstetrical and perinatal factors linked to longitudinal microbiota analysis.

In addition to these microbial factors, other potentially contributing factors need to be acknowledged. For example, it is tempting to speculate that pre-eclampsia or intra-uterine growth restriction might have caused suboptimal antenatal Doppler ultrasound features which in turn may have led to the decision to perform an emergency caesarean section. In these specific cases, administration of antibiotics is often not initiated since an intrauterine infection is not suspected. Therefore, merely the fetal Doppler ultrasound abnormalities [21] and not the absence of administration of antibiotics within 24 h after birth may have contributed to the increased association with the development of NEC.

In contrast to the univariate analysis, endurance of a septic episode in the entire period prior to NEC was not associated with an increased risk of developing NEC in

the multivariable analysis. Interestingly, infants developing NEC had increased odds of developing sepsis within 24 h adjacent to clinical NEC diagnosis. Presumably, the release of pro-inflammatory cytokines and endotoxemia during NEC onset causes failure of the mucosal barrier function, ultimately leading to bacterial translocation and concurrent bloodstream infection [22].

In the literature, the concept of slow advancements in enteral feeding volumes and consequently an increased number of parenteral nutrition days demonstrated no significant alterations in the overall incidence of NEC except in infants with a birth weight <750 g [23]. In contrast to the number of parenteral feeding days, the velocity of enteral feeding volume expansion during the first postnatal week was not associated with the development of NEC. Although enteral feeding advancements in the first week did not differ between the 2 groups, we hypothesize that enteral feeding intolerance present after the first postnatal week is more prevalent in infants developing NEC, explaining the observed increase in the cumulative number of parenteral feeding days.

In addition, the administration of predominantly formula feeding was associated with an increased risk of developing NEC. Both the presence of intact bovine proteins leading to intestinal inflammation [24] and the absence of human milk with associated protective properties [25] contribute to this increased risk. During this study, two centers started with supplementation of donor milk in cases where the production of the own mother's milk was insufficient. In the current study, we did not differentiate between donor milk and the own mother's milk. However, since supplementation with either donor milk or formula yielded similar short-term outcomes such as NEC [26], we hypothesize that the observed protective effect of the own mother's milk may potentially be higher than observed.

A strength of the current study is the prospective design with detailed daily data collection at 9 centers allowing us to match each case with 5 controls. By not including center of birth in the matching procedure, inter-center differences in medical policies (e.g., regarding feeding practices or types of antibiotics), potentially causing an increased risk of developing NEC, would possibly have been identified in the current study. This study also has several limitations. Firstly, to allow for adequate comparisons in the case-control cohort, control infants were matched based on their postnatal age. Consequently, all control infants at least survived their matched case and, therefore, may not have been an adequate representation of the overall population. Secondly, since the follow-up period was limited, infants developing NEC after this follow-up period may hypothetically have been allocated to the control group. However, information concerning the development of NEC was collected for the entire NICU admission period. None of the selected controls were transferred before the corrected postmenstrual age (PMA) of 32 weeks. Since the majority of NEC cases occur before PMA of 30 weeks, the risk that a control infant developed NEC after PMA of 32 weeks and, thus, was incorrectly allocated to the control group is relatively low [14]. Thirdly, the current study is limited by the absence of detailed obstetric data, such as prenatal exposure to corticosteroids, pre-eclampsia, antenatal Doppler ultrasound features, chorioamnionitis, and intrapartum signs of infection. However, by including retrospectively collected variables to data which have been collected in a prospective manner would potentially result in biased outcomes and conclusions. Future studies should focus on the collection of both clinical and obstetric data while simultaneously performing longitudinal microbiota analyses to explore any causality between early administered antibiotics and the development of NEC. Furthermore, this study dealt with missing enteral and parenteral feeding practice values in approximately 10% of all included cases. Since all these cases originated from 2 centers, these values were not missing randomly and could, thus, not be statistically corrected for. Lastly, although the detailed manner of data collection allowed for the inclusion of a substantial number of different clinical variables, this also resulted in an increased risk of false-positive discoveries (type I error) due to multiple testing.

Future studies should focus on validating the current study results by using an external cohort. Based on these findings, a prediction model may be constructed, allowing for the early identification and selection of those infants at risk of developing NEC. Subsequently, interventional studies may be performed to explore causality between the development of NEC and the currently identified clinical variables associated with NEC.

In conclusion, in this multicenter prospective cohort study multiple independent risk factors associated with the development of NEC were identified: predominate formula feeding and the cumulative number of parenteral feeding days. This is the first prospective multicenter study describing that exposure to antibiotics initiated within 24 h after birth is associated with a decreased risk of developing NEC. This observation seems to underline the increasing notion that early intestinal colonization might play a pivotal role in the pathogenesis of NEC and opens avenues towards the development of microbiota-based preventive strategies in order to reduce NEC incidences.

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Statement of Ethics

This study was approved by the local Institutional Review Boards of all 9 medical centers (2014.386 amendment A2016.363). Informed consent was obtained from all parents.

Disclosure Statement

The authors declare no conflicts of interest.

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